

**IN THE UNITED STATES PATENT AND TRADEMARK**

Inventor : Britta Hardy and Avraham Novogrodsky  
Serial No. : 08/380,857  
Filed : January 30, 1995  
Title : *"Immuno-Stimulatory Monoclonal Antibodies"*

Group Art Unit : 1806

Honorable Commissioner of Patents and Trademarks

**SUPPLEMENTAL DECLARATION**

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AUG 22 1997

GPO

S I R :

1. I am one of the inventors in Patent Application No. 08/380,857 (hereinafter: *the "application"*), and I am the same Declarant who submitted a Declaration to the U.S. Patent Office on February 27, 1997 (hereinafter: *"the First Declaration"*).

2. In the First Declaration mention is made to a monoclonal antibody referred to as *"BAT mAb"*. This is the same monoclonal antibody as the BAT-1 monoclonal antibody referred to in the application.

3. In the First Declaration, experiments are reported which were made in human-mouse xenograft models, consisting of engrafted human tumors in immunodeficient mice (SCID and nude) (hereinafter: *"human-mice xenograft models"*). Human-mice xenograft models are acceptable models in the field of drug develop-

ment for human use, and particularly such cancer models are acceptable in the development of anti-cancer drugs for human use. Furthermore, such models are acceptable as being predictive of the activity of drugs in humans. In view of their predictive value, experiments in human-mice xenografts, are acceptable as being a last step before entering into human clinical trials with a developed drug. Accordingly, the human-mice xenograft models used in my experiments with the BAT-1 monoclonal antibodies, are predictive regarding the activity of BAT-1 monoclonal antibodies in humans.

4. I further declare that all statements made herein of my own knowledge are true, that all made statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment or both, under Section 100 of Title 18 of the United States Code and that such wilful statements may jeopardize the validity of the application or any patent issued thereon.

Dated this 23 day of July, 1997

B. Hardy  
BRITTA HARDY

18 '97 13:33  
 No. Records Requested Available Copy  
 #1: 3146 SCID  
 #2: 470953 MICE  
 #3: 2433 SCID MICE  
 #4: 2843 XENOGRAFT  
 #5: 82 #3 and #4  
 #6: 692187 HUMAN  
 #7: 76 #5 and #6  
 #8: 305673 TUMOR  
 50 #7 and #8

P.4

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MEDLINE EXPRESS (R) 1992-1996

1 of 5  
Marked Record

I: Doxorubicin encapsulated in sterically stabilized liposomes is superior to free drug or drug-containing conventional liposomes at suppressing growth and metastases of human lung tumor xenografts.  
 U: Sakakibara-T; Chen-FA; Kida-H; Kunieda-K; Cuenca-RE; Martin-FJ; Bankert-RB  
 D: Department of Molecular Immunology, Roswell Park Cancer Institute, Buffalo, New York 14263, USA.  
 O: Cancer-Res. 1996 Aug 15; 56(16): 3743-6  
 This Journal is Owned by this Library. Holdings According to  
 Call Number: 1972 v.32-  
 SSN: 0008-5472

A: ENGLISH

B: Liposomes containing polyethylene glycol-derivatized phospholipids are able to evade the reticuloendothelial system and thereby remain in circulation for prolonged periods. We report here that doxorubicin encapsulated in these sterically stabilized liposomes (S-DOX) suppresses the growth of established human lung tumor xenografts in severe combined immunodeficient (SCID) mice and inhibits the spontaneous metastases of these tumors. The enhanced therapeutic efficacy of S-DOX compared to free doxorubicin was demonstrated in two independent human/mouse models. In the first model, S-DOX inhibited the growth of a human non-small cell lung tumor xenograft established orthotopically in the lungs of SCID mice. Treatment of these mice with S-DOX, but not with free drug, suppressed the growth of the tumor in the lung, prevented metastasis from the lung, and enhanced survival percentage. In another model, the human lung tumor is engrafted into gonadal fat pad of SCID mice. Human tumor xenografts grow floridly in this site of engraftment, and the tumor spreads from this primary site into the peritoneal cavity and subsequently reaches the liver and lung. In this model, free drug suppressed the growth of the primary tumor but had no effect upon the subsequent spread of the tumor into the peritoneal cavity, liver, and lung. In contrast, treatment of the tumor-bearing mice with S-DOX (but not with doxorubicin in conventional liposomes) suppressed the tumor spread to the peritoneal cavity, completely arrested metastasis to the liver and lung, and suppressed the growth of the primary tumor xenograft. This report provides the first evidence that antitumor drugs delivered by sterically stabilized liposomes can arrest the metastasis of human tumor xenografts.  
 IN: 96328097

MEDLINE EXPRESS (R) 1992-1996

2 of 5

Be Available Copy

TI: In vitro and in vivo anti-tumour effects of a humanised monoclonal antibody against c-erbB-2 product.

AU: Tokuda-Y; Ohnishi-Y; Shimamura-K; Iwasawa-M; Yoshimura-M; Ueyama-Y; Tamaoki-N; Tajima-T; Mitomi-T

AD: Department of Surgery, Tokai University School of Medicine, Kanagawa, Japan.

SO: Br-J-Cancer. 1996 Jun; 73(11): 1362-5

This Journal is Owned by this Library. Holdings According to

Call Number: 1972 v.26-

ISSN: 0007-0920

LA: ENGLISH

AB: The c-erbB-2 product is thought to be a unique and useful target for antibody therapy of cancers overexpressing the c-erbB-2 gene. In vitro and in vivo anti-tumour effects of a humanised antibody against the extracellular domain of the c-erbB-2 gene product, rhu4D5, were examined. Rhu4D5 was less effective than its murine counterpart, mu4D5, for the direct antiproliferative activity against the c-erbB-2-overexpressing SK-BR-3 cell line. In vivo treatment of severe combined immunodeficient (SCID) mice carrying the c-erbB-2-overexpressing 4-1ST human gastric carcinoma xenograft with rhu4D5 revealed that the recombinant protein had potent anti-tumour activity. Furthermore, cytotoxicity of human peripheral blood mononuclear cells against 4-1ST was significantly augmented with rhu4D5, but not with mu4D5. These results indicate that rhu4D5 might perform better in patients than predicted from preclinical studies.

AN: 96249051

MEDLINE EXPRESS (R) 1992-1996

3 of 5  
Marked Record

TI: Establishment of a human B-CLL xenograft model: utility as a preclinical therapeutic model.

AU: Mohammad-RM; Mohamed-AN; Hamdan-MY; Vo-T; Chen-B; Katato-K; Abubakr-YA; Dugan-MC; al-Katib-A

AD: Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201, USA.

SO: Leukemia. 1996 Jan; 10(1): 130-7

ISSN: 0887-6924

LA: ENGLISH

AB: Chronic lymphocytic leukemia (CLL), a proliferative disease of mature looking B lymphocytes, is the commonest leukemia in western countries. It remains incurable by available treatment modalities. We report on the establishment of a permanent, EBV-negative, B-CLL line (WSU-CLL) from the peripheral blood of a patient with CLL. The cells grow as suspension in liquid culture, express IgG lambda and other B cell markers and show lg heavy and light gene rearrangements. Karyotypic analysis shows 45,X,del(3)(p14;p24),t(4;12;12)(q31;q22;p13), t(5;12)(q31;p13), add(16)(q24)x2, t(18;21)(q12;p12). WSU-CLL forms colonies when grown on soft agar. A xenograft model was established by injecting the WSU-CLL cells subcutaneously (s.c.) in severe combined immune deficient (SCID) mice. When the s.c. tumor was transplanted in vivo to other SCID mice, the success rate was 100% with a doubling time of 7.3 days. The CLL-SCID xenograft model was used to test the efficacy of selected standard chemotherapy drugs and new therapeutic agents against WSU-CLL. The cell line and the xenograft described can be used as a model to facilitate the development of new therapeutic agents against CLL in man.

AN: 96145222



(Y)

I: The urokinase inhibitor p-aminobenzamidine inhibits growth of a P. Enan prostate tumor in SCID mice.  
 U: Billstrom-A; Hartley-Asp-B; Lecander-I; Batra-S; Astedt-B  
 D: Pharmacia Oncology Immunology, University Hospital, Lund, Sweden.  
 O: Int-J-Cancer. 1995 May 16; 61(4): 542-7

This Journal is Owned by this Library. Holdings According to

Call Number: 1972 v.9-

SSN: 0020-7136

A: ENGLISH

B: Malignant cells possess a high degree of proteolytic activity in which the plasminogen activator system plays an important role. An increased expression of urokinase type plasminogen activator (uPA) is of significance for degradation of the extracellular tumor matrix, facilitating invasiveness and growth. Inhibition of the active site of uPA makes it possible to evaluate the significance of uPA in tumor growth. We report here experiments on a PA-producing human prostate xenograft (DU 145) using a competitive inhibitor of uPA, p-aminobenzamidine. In vitro experiments with DU 145 cells showed that p-aminobenzamidine caused a dose-dependent inhibition of uPA activity. DU 145 cells were inoculated s.c. in SCID mice and, once tumors were established, treatment with p-aminobenzamidine added to drinking water was started and lasted for 23 days. Mice receiving 250 mg/kg/day of p-aminobenzamidine showed a clear decrease in tumor-growth rate compared to the non-treated mice, resulting in 64% lower final tumor weight. In addition, uPA-antigen levels in the membrane fractions of DU 145 tumors from p-aminobenzamidine-treated mice were found to be decreased by 59%. We also show that p-aminobenzamidine has an anti-proliferative effect in cell culture at low cell number, correlating with a dose-dependent decrease in uPA production. In conclusion, we show that a low-molecular-weight uPA-inhibitor, p-aminobenzamidine, has a growth-inhibitory effect on a solid uPA-producing tumor.

IN: 95279003

MEDLINE EXPRESS (R) 1992-1996

5 of 5  
Marked Record

II: Dexamethasone reduces the interstitial fluid pressure in a human colon adenocarcinoma xenograft.

U: Kristjansen-PE; Boucher-Y; Jain-RK  
 D: Edwin L. Steele Laboratory, Harvard Medical School, Massachusetts General Hospital, Boston 02114.  
 O: Cancer-Res. 1993 Oct 15; 53(20): 4764-6

This Journal is Owned by this Library. Holdings According to

Call Number: 1972 v.32-

ISSN: 0008-5472

LA: ENGLISH

AB: The effect of dexamethasone on interstitial hypertension was evaluated in a human colonic adenocarcinoma. Two weeks after transplantation of the tumor line LS174T into SCID mice, recipients with tumors > 8.5 mm in diameter received one daily injection i.p. on days 1-4, at five different doses in the range of 0.3-30 mg/kg. Controls received saline. The interstitial fluid pressure (IFP) was determined in all tumors pretherapeutically on days 1, 4, and 7. A total of 68 tumors were examined, and in an additional group of 22 mice, the effect of 4-day dexamethasone therapy on blood pressure was evaluated. In the 3-, 10-, and 30-mg/kg dose groups a significant reduction in IFP was found, comparing treated versus controls and individual measurements from day 1 versus day 4. No

..... AUG 18 '97 13:36 affect the IFP. The systemic blood pressure was  
whereas 0.3 mg/kg did not affect the IFP. The systemic blood pressure was  
slightly increased by dexamethasone therapy, and treatment related  
changes in tumor sizes were observed. Our findings indicate that the reversible  
decrease in tumor IFP by dexamethasone is an effect of a reduced microvascular  
permeability and vascular hydraulic conductivity in the tumors.  
I: 94006268

6

**CURRICULUM VITAE**

**Date and Place of birth:** April 23, 1946 , Russia  
**Identity Number** 0450713-3  
**Emigrated to Israel:** May 1949  
**Citizenship:** Israeli  
**Marital Status:** Married + two children  
**Home Address:** Eliyahu Hakkim 6  
Tel Aviv 69120  
**Home Telephone:** (03) 6423089  
**Work Address** Head , Unit of Cell Immunology  
Felsenstein Medical Research Center  
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Petah Tikva 49100  
**Work Telephone:** (03)-9376782 (Office)  
(03)-9376625/6 (Lab)  
**Fax No.** 972-3-9211478

**A. EDUCATION**

- 1959-1963 Secondary School "Kol Israel Haverim"  
(Alliance), Ramat Aviv, Tel Aviv
- 1963-1967 *B.Sc. in Microbiology*, Bar Ilan University, Ramat Gan,  
Studied Microbiology and Biochemistry
- 1967-1969 *M.Sc. in Microbiology* (with distinction)  
Bar Ilan University: Thesis entitled:  
The effect of FSH on RNA synthesis in immature mice ovaries  
Supervisor: Prof. B. Lunenfeld.
- 1970-1975 *Ph.D.* student at the Feinberg Graduate School  
and the Dept. of Biological Ultrastucture at the  
Weizmann Institute of Science , Rehovot, Israel  
Supervisor: Prof. David Danon.
- Oct. 1975 *Received Ph.D.* degree at the Weizmann Institute  
of Science, Rehovot. Thesis entitled:  
Structural and functional differences in macrophages of mice  
deficient in antibody production.

## **B. FURTHER STUDIES**

- Sept. 1976-July 1979 Post Doctoral Fellow in Hematology, Stanford University,  
School of Medicine, Dept. of Medicine, CA., USA  
Research on "The role of spectrin and actin in the red blood  
cell membrane."  
Supervisor: Prof. Stanley Schrier, Head of Hematology.
- Awarded: Chaim Weizmann Fellowship for Post Doctoral Studies in the  
U.S.A.
- Awarded: Fellow of Hematology. Stanford University Medical School.
- Oct. 1984-July 1985 Visiting Research Fellow at Stanford University Medical School  
Dept of Pediatrics.  
Research on monoclonal antibodies to endothelial cells.  
Dean's Fellowship  
Awarded: Fellow of Medicine

## **C. EMPLOYMENT**

- 1968-1969 Research assistant at the Bar Ilan University, Ramat-Gan, Israel  
Dept. of Microbiology
- Sept. 1975-Sept 1976 Research Scientist, Department of Biological Ultrastructure  
Weizmann Institute of Science, Rehovot, Israel
- Sept. 1976-July 1979 Post doctoral Fellow at Stanford University School of Medicine  
Department of Medicine, Hematology Division,  
Stanford, CA., USA  
Head, Prof. S.L. Schrier
- Sept. 1979-1980 Dept. of Chemical Immunology  
The Weizmann Institute of Science, Rehovot, Israel  
Laboratory of Prof. Edna Mozes
- Jan 1980-Sept. 1985 Rogoff Medical Research Institute, Beilinson Medical Center  
Petah Tivka, Israel.  
Head of Lipidosis Unit for Research and diagnosis of Gaucher  
and Niemann Pick diseases.  
Received Tenure in Kupat Holim
- Oct. 1985- July 1986 Stanford University, Medical Center, Dept. of Pediatrics, CA., USA  
Dean's Fellowship



Sept. 1985-July 1988 Rogoff Medical Research Institute, Beilinson Medical Center,  
Petah Tikva, Israel. Head of Unit for development use of monoclonal  
antibodies in research and treatment of disease.

Sept. 1988-July 1989 IDEC Pharmaceutical Corp., Mountain View, CA, USA.  
Research on shared idiotypes in B lymphoma and production of  
anti-idiotypes for the treatment of B lymphoma patients.

Sept. 1989-June 1993 Rogoff Medical Research Institute, Beilinson Medical Center., Israel  
The Laboratory was moved to Felsenstein Medical Research. Center,  
Beilinson Campus, Petah Tikva , Israel

June 1993-present Head of Cell Immunology Unit

## List of Publications

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Cytochemical Findings in Lymphocytes in blood culture.  
Harefuah, 1967, V.LXXIII n. 6. 193-195.
2. A. Eshkol, **B. Hardy** and C. Pariente-Coriat.  
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4. **B. Hardy**, A. Globerson and D. Danon.  
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5. **B. Hardy**, E. Mozes and D. Danon.  
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Immunology, 1976, 30: 261-266.
6. **B. Hardy**, E. Skutelsky, A. Globerson and D. Danon.  
Ultrstructural differences between macrophages of newborn and adult mice.  
J. Reticulendothel. Soc., 1976, 19: 291-299.
7. E. Skutelsky and **B. Hardy**.  
Regeneration of plasmalemma and surface properties of macrophages.  
Exp. Cell. Res., 1976, 101: 337-345.
8. **B. Hardy** and E. Mozes.  
Expression of T-cell suppressor activity in the immune response to a T-independent synthetic polypeptide in newborn mice.  
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9. **B. Hardy** and S.L. Schrier.  
The role of spectrin in erythrocyte ghost endocytosis.  
Biochem.Biophys.Res.Comm., 1978, 84:1153-1161.
10. S.L. Schrier, **B. Hardy**, K. Bensch, I. Junga and J. Krueger.  
Red blood cell membrane storage lesion.  
Transfusion, 1979, 19: 158-165.

11. S.L. Schrier, **B. Hardy** and K.G. Bensch.  
Endocytosis in erythrocytes and their ghosts.  
In: Normal and abnormal red cell membranes. Eds. Lux, SE, Marchesi, VT, Fox, CF.  
New York. Alan R. Liss, 1979, p. 437-449.
12. **B. Hardy**, K.G. Bensch and S.L. Schrier.  
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J. Cell. Biol., 1979, 82: 654-663.
13. S.L. Schrier, **B. Hardy**, I. Junga and L. Ma.  
Actin activated ATPase in human red cell membranes.  
Blood, 1981, 58: 953-962.
14. **B. Hardy**, J.M. Loew, I. Melchers, D. Charron and S.L. Schrier.  
Monoclonal antibodies to red cell cytoskeletal proteins.  
Archives of Biochemistry and Biophysics, 1982, 213: 334-337.
15. **B. Hardy**, J. Hoffman, and A. Neri.  
Niemann-Pick carrier detection in lymphocytes.  
Biomedicine, 1982, 36: 372-375.
16. **B. Hardy**, J. Hoffman and Z. Ossimi.  
Immunological and isoelectric focusing study of b glucocerebrosi- dase from  
normal and Gaucher disease.  
Biochem. Biophys. Res. Comm., 1984, 120: (2) 325-332.
17. C. Clayberger, T. Uyehara, **B. Hardy**, K. Eaton, M. Karasek and A.M. Krensky.  
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T lymphocyte response to endothelial cells.  
J. Immunol., 1985, 135 (1): 12-18.
18. C. Clayberger, B. Dyer, B. McIntyre, T.D. Koller, **B. Hardy**,  
P. Parham, L.L. Lanier and A.M. Krensky.  
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cell-associated antigen.  
J. Immunol., 1986, 136: 1537-1541.
19. **B. Hardy**, B. Teitelman-Weisman, S. Chazan and A. Neri.  
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Biomedicine, 1987, 41: 40-44
20. **B. Hardy**, Y. Hoffman and S. Chazan.  
Use of monoclonal antibodies in the study of Gaucher disease.  
Harefuah, 1987, V. CXIII n.9 204-206

21. **B. Hardy**, D. Dotan and A. Novogrodsky  
A monoclonal antibody to human B lymphoblastoid cells  
activates human and murine T lymphocytes.  
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22. D. Huminer, **B. Hardy**, S. Pitlik.  
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23. **B. Hardy**, I. Yampolski, R. Kovjazin, M. Galli and A. Novogrodsky.  
A monoclonal antibody against a human B lymphoblastoid cell line  
induced tumor regression in mice.  
Cancer Research 1994, 54: 5793-5796.
24. **B. Hardy**, M. Galli, E. Rivlin, L. Goren, and A. Novogrodsky  
Activation of human an lymphocytes by a monoclonal antibody to B  
lymphoblastoid cells; molecular weight and distribution of binding protein.  
Cancer Immunology Immunotherapy. 1995,40(6) 376-382
25. L. Wasserman, A. Neri, Y. Manor, B. Kaplan, M. Galli and **B. Hardy**.  
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characterization of the antigen.  
J. of Cancer Research and Clinical Oncology, 1995, 121 (7) 387-392
26. M. Shohat, **B. Hardy**, S. Mannheimer, B. Fisch and B. Shohat  
A new method for isolation of human antisperm antibodies.  
ANDROLOGIA 1996, 28 275-279
27. **B. Hardy**, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky  
Immune Stimulatory and anti-tumor properties of anti-CD3 and BAT monoclonal  
antibodies: A comparative study. Human Antibodies and Hybridomas . In  
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28. **B. Hardy**, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky  
A lymphocyte-Activating monoclonal antibody induces regression of human tumors  
in SCID mice. Submitted

### Short Communications

1. D. Friedman, **B. Hardy**, D. Danon and A. Globerson.  
Immunological status of the newborn mouse spleen.  
Israel. J. Med. Sci. 1974, 10, 1179.
2. **B. Hardy**, A. Globerson and D. Danon.  
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Proc. 4th Congr. Internat. Soc. Hemat., 1974, p. 414.
3. **B. Hardy** and D. Danon.  
A microscopic study comparing the phagocytic function of  
newborn and adult mouse macrophages.  
Proc. 10th Internat. Congr. of Geront. 1975, V. 2 No:39.
4. **B. Hardy**, E. Mozes and D. Danon.  
Comparison of the ability of newborn mice to produce antibodies  
to a T-independent and T-dependent synthetic polypeptide.  
Isr. J. Med. Sci. 1975, 11: 1377.
5. **B. Hardy** and E. Mozes.  
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Scand. J. Immunol. 1977, 6, 705.
6. E. Mozes and **B. Hardy**.  
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7. **B. Hardy** and S.L. Schrier.  
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Proc. 20th Meeting of American Soc. Hematol. San Diego, Ca.
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10. **B.Hardy**, K.G. Bensch and S.L. Schrier.  
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Blood, 1979, 54, 25a. 22nd Ann. Mtg. American Soc. Hematol  
Phenix, Ari.
12. S.L. Schrier and **B. Hardy**.  
Human erythrocyte actin activated Mg-ATPase.  
Blood, 1979, 54, 31a.  
Ann. Mtg. American Hematol. 1979, Phenix, Ari.
13. J. Hoffman, A. Neri and **B. Hardy**.  
Distribution of sphingomyelinase activity in white cell populations.  
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14. **B. Hardy**, J. Hoffman and I. Ossimi.  
Immunological studies on m-glucocerebrosidase isozymes from spleens of normal and Gaucher variants.  
Proc. The 1st Joint Meet. of the Isr. Soc. for Life Sci. (Jerusalem) Oct. 1983, C-121.
15. **B. Hardy**, Z. Ossimi, K.H. Stenzel and A. Novogrodsky.  
Monoclonal antibodies inhibiting accessory function of human B lymphoblastoid cell lines.  
Israel Immunological Society 16th Cong. May 1986, p. 19.
16. **B. Hardy**, M. Galli, E. Rivlin, E. Pras and A. Novogrodsky.  
Activation of T lymphocytes by monoclonal antibodies to B lymphoblastoid cells.  
Israel J. Med. Science, 1991.
17. M. Shohat, B. Shohat, **B. Hardy**, S. Manheimer, A. Stein and B. Fisch.  
A new method for isolation of pure human antisperm antibodies.  
Ann. Meet. of the Israeli Soc. of Reproduction. April 1993, Israel p. 150.
18. **B. Hardy**, R. Kovjazin, A. Raiter, N. Ganor and A. Novogrodsky  
A novel monoclonal antibody with immune -stimulatory and anti-tumor properties.  
Human Antibodies and Hybridomas 1996 7 (2) 54 (abstract)